FEBS Letters 585 (2011) 2223–2228

journal homepage: www.FEBSLetters.org

Identification of a novel DNA element that promotes cell-to-cell transformation in *Escherichia coli*

Rina Sobue, Naomi Kurono, Rika Etchuya, Sumio Maeda *

Graduate School of Humanities and Sciences, Nara Women's University, Kitauoya-Nishimachi, Nara 630-8506, Japan

ARTICLE INFO

Article history:
Received 18 April 2011
Revised 16 May 2011
Accepted 18 May 2011
Available online 26 May 2011

Edited by Renee Tsolis

Keywords:
Cell-to-cell transformation
DNA uptake sequence
Horizontal gene transfer
pHSG299
R6-5
Escherichia coli

ABSTRACT

Recently, we discovered a novel phenomenon, "cell-to-cell transformation" by which non-conjugative plasmids are transmitted horizontally in co-cultures of *Escherichia coli* F⁻ strains. In this study, we aimed to identify the DNA element responsible for the high cell-to-cell transformability of pHSG299. By transplanting pHSG299 DNA fragments into pHSG399, a plasmid showing low transformability, we discovered that a specific 88 bp fragment of pHSG299 significantly promoted pHSG399 transformability. Although several short motif-like repetitive sequences (6–10 bp) were present in the 88 bp sequence, no known DNA motifs were recognized, suggesting that this 88 bp sequence (cell-to-cell transformation promoting sequence, CTPS; Accession number: AB634455) is a novel DNA element.

1. Introduction

Bacterial cells acquire new genetic traits by horizontal DNA transfer to adapt themselves to their environment and improve their chances of survival [1–3]. Genetic transformation is one of the mechanisms by which cells take up extracellular naked DNA released from other cells [4,5].

In most known systems, DNA uptake by transformation is not sequence-specific [4,5]. However, in some Gram-negative bacteria such as *Neisseria* species and *Haemophilus influenzae* efficient uptake occurs only when a specific short sequence is present [5–8]. The sequence motifs that are required for efficient uptake, i.e., DNA uptake sequence (DUS) and uptake signal sequence (USS), have been identified for *Neisseria* sp. (5′-(AT)GCCGTCTGAA-3′) and *H. influenzae* (5′-AAGTGCGGT-3′) [5–8].

In *Escherichia coli*, artificial transformation by the calcium chloride ($CaCl_2$) [9,10] or other methods [11,12] is well known as a common laboratory technique, but natural transformation is poorly understood. Several reports [13–18] including ours [19–21] demonstrated that *E. coli* can develop modest genetic competence for transformation under conditions occurring in usual natural and human environments.

In recent studies, we found that horizontal transfer of non-conjugative plasmids occurs spontaneously in F⁻ *E. coli* cell-mixed cultures [22,23]. Subsequently, we demonstrated that this plasmid transfer occurs because of a specific type of natural transformation in which DNA derived from co-cultured cells is essential [24]. This transformation was DNase-sensitive and clearly distinguishable from conjugation by a filter-mediated plasmid transfer experiment [24]. Accordingly, we termed this new type of transformation as "cell-to-cell transformation" [24]. Because promoting conditions for cell-to-cell transformation did not promote simple natural and artificial transformations with purified plasmid DNA, it was suggested that the mechanism of cell-to-cell transformation differs from those of the known types of transformation in *E. coli* [24].

In this study, to further elucidate the mechanism of the cell-to-cell transformation in *E. coli*, we investigated the features of the specific plasmid pHSG299 [25], which shows a high level of transformability. We present data obtained from transplantation and deletion analyses of the plasmid sequence, demonstrating the presence of a novel functional DNA sequence that can specifically promote cell-to-cell transformation in *E. coli*.

2. Materials and methods

2.1. E. coli strains, plasmids, and materials

The following *E. coli* strains and plasmids were obtained from the "National BioResource Project (NIG, Japan): *E. coli* (http://www.

Abbreviation: CTPS, cell-to-cell transformation promoting sequence

* Corresponding author. Fax: +81 742 20 3967.
E-mail address: smaeda@cc.nara-wu.ac.jp (S. Maeda).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.05.040

shigen.nig.ac.jp/ecoli/strain/top/top.jsp): DH5 (F^-, *deoR, recA1, endA1, hsdR17(rK⁻, mK⁺), supE44, λ⁻, thi-1, gyrA96, relA1*); CAG18439 [26] (MG1655 derivative; F^-, λ^-, *lacZ118(Oc), lacI3042::Tn10(tet^r), rph-1*); pHSG299 [25] (*kan^r*, a pMB1-derived high-copy cloning vector that lacks the *tra*, *mob*, and *nic-bom* regions; GenBank ID: M19415); pHSG399 [25] (*cam^r*, a pMB1-derived high-copy cloning vector similar to pHSG299; GenBank ID: M19087); and pUC19 (lacking the *tra*, *mob*, and *nic-bom* regions) [12]. PCR enzyme KOD FX was obtained from Toyobo (Osaka, Japan). Ampicillin (amp), tetracycline (tet), chloramphenicol (cam), polyethylene glycol (PEG; molecular mass = 8000) and Luria–Bertani powder (LB; Lennox) were obtained from Sigma (St. Louis, MO, USA). Tryptic Soy Broth was obtained from Becton, Dickinson (Franklin Lakes, NJ, USA). Distilled water (DNase- and RNase-free; molecular biology grade) and kanamycin (kan) were purchased from Invitrogen (Carlsbad, CA, USA), and a nylon66 membrane filter (pore size: 0.45 μm; Biodyne A) was obtained from Pall (Port Washington, NY, USA). Agar powder (guaranteed-reagent grade) and other general reagents were purchased from Wako (Osaka, Japan).

2.2. Plasmid construction

Plasmids were constructed as follows: the PCR enzyme KOD FX was used for plasmid-constructing PCR according to the reaction conditions recommended by the manufacturer. The primers used are shown in Supplementary Tables S1–S3. Modified or inserted sequences in the constructed plasmids were confirmed by DNA sequencing.

pHSG399-F1–F6: By comparing complete sequences of pHSG299 and pHSG399, pHSG299-specific sequences were identified and divided into six fragments of approximately 180–370 bp length. These fragments were named F1–F6 and amplified by PCR using corresponding primers (Supplementary Table S1) in which a *BamH*I or *Eco*RI site was added to the 5′ ends. PCR reactions were conducted by initial denaturation at 94 °C for 4 min, 25 cycles of 30 s at 98 °C, 40 s at 65 °C (−0.1 °C per cycle), 50 s at 72 °C, and final extension at 72 °C for 5 min. The resulting PCR products were cut using *BamH*I and *Eco*RI and cloned into the *BamH*I–*Eco*RI site in the multicloning site of pHSG399.

pHSG299ΔF6: A 2482 bp fragment of pHSG299, from which the F6 fragment was removed, was constructed by PCR using two primers (Supplementary Table S1) immediately adjacent to the F6 sequence. *Bgl*II sites were added to the 5′ ends of these primers. The PCR reaction was conducted by initial denaturation at 94 °C for 4 min, 40 cycles of 30 s at 98 °C, 40 s at 40 °C (−0.1 °C per cycle), 180 s at 70 °C, and final extension at 72 °C for 5 min. The resulting PCR product was cut using *Bgl*II and self-ligated, producing pHSG299ΔF6.

pUC19-tet-F6: The *Eco*RI–*Mro*I short fragment containing the *tet^r* gene of pBR322 was cloned into the *Eco*RI–*Cfr*9I site of pUC19, producing pUC19-tet. The F6 sequence of pHSG299 was amplified by PCR using primers (Supplementary Table S1) containing *Pst*I and *Xba*I sites at their 5′ ends. The PCR reaction was conducted by initial denaturation at 94 °C for 4 min, 26 cycles of 30 s at 98 °C, 40 s at 65 °C (−0.1 °C per cycle), 180 s at 72 °C, and final extension at 72 °C for 5 min. The PCR product was cut using *Pst*I and *Xba*I and cloned into the *Pst*I–*Xba*I site in the multicloning site of pUC19-tet, producing pUC19-tet-F6.

pHSG399-F6 deletion and base substitution mutants: A series of deletion and base substitution mutants of the F6 sequence of pHSG399 were constructed by PCR using pHSG399-F6, its derivatives, and pHSG399 as the template and corresponding primers (Supplementary Table S2) containing additive *Kpn*I or *Bgl*II sites at their 5′ ends. The PCR reaction was conducted by initial denaturation at 94 °C for 4 min, 40 cycles of 30 s at 98 °C, 40 s at 40–51 °C (−0.1 °C per cycle), 150 s at 68 °C, and final extension at 68 °C for 5 min. Annealing temperatures were altered between 40–51 °C (initial temperature) according to the primer set. The PCR product was cut using *Kpn*I or *Bgl*II and self-ligated, producing the required deletion plasmids.

pHSG399-Tsen's sequences: pHSG399 derivatives with Tsen's sequences [16] were constructed by PCR using pHSG399 as the template and corresponding primers (Supplementary Table S3) containing Tsen's sequences and additive *Kpn*I sites at their 5′ ends. The PCR reaction was conducted by initial denaturation at 94 °C for 4 min, 40 cycles of 30 s at 98 °C, 40 s at 50 °C (−0.1 °C per cycle), 150 s at 68 °C, and final extension at 68 °C for 5 min. The PCR product was cut using *Kpn*I and self-ligated, producing the required plasmids.

2.3. Cell-to-cell transformation experiments

Cell-to-cell transformation experiments were performed in a colony-biofilm system using DH5 as plasmid donor cells and CAG18439 as plasmid recipient cells, as described previously [22–24]. The occurrence of cell-to-cell transformation was detected by the appearance of double-resistant transformants [tet^r (CAG18439) and kan^r (pHSG299 and its derivative) or cam^r (pHSG399 and its derivatives)]. The transformation frequency was calculated as the ratio of the number of transformants to the estimated number of recipient cells, which was taken as half of the total number of cells in each sample. The total number of cells in each sample was deduced from the OD_{600} value of the cell suspension immediately before plating.

In the cell-to-cell transformation experiments, to exclude a possibility of the occurrence of unexpected conjugation by accidentally contaminated conjugative plasmids, we adopted only specific non-conjugative plasmids (pHSG299, pHSG399, and pUC19) that do not possess the *tra*, *mob*, and *nic-bom* regions in combination with F^- strains. Particularly, lacking of the nic-bom sequence completely abolishes passive conjugation of non-conjugative plasmids directed by conjugative plasmids coexisting in the same cell [12]. In addition, in order to confirm the absence of unexpected contamination of F plasmid in the strains used, we analyzed the strains by PCR using primers for *traI* and *traD* specific to F plasmid, and obtained a result that show absence of F plasmid (data not shown).

2.4. Natural transformation experiment

Natural transformation experiments were performed in a colony biofilm as follows: CAG18439 cells were precultured, cultured, and plated following the protocol used for cell-to-cell transformation experiments, except that plasmid DNA (10 μg), which was purified by an alkali method and phenol–chloroform extraction, was added along with CAG18439 at the beginning of culture. The transformation frequency was calculated as the ratio of the number of transformants to the number of recipient cells.

2.5. Artificial transformation experiments

The $CaCl_2$ [9,10] and PEG methods [11] were performed as described previously [24]. The transformation frequency was calculated as the ratio of the number of transformants to the number of recipient cells.

3. Results

3.1. pHSG299 shows much higher activity than pHSG399 in cell-to-cell transformation

The activity of pHSG299 in cell-to-cell transformation was compared with that of pHSG399 [25]; both plasmids are similar and

contain the same replication origin (pMB1) and multicloning site. As shown in Fig. 1A, despite using the same donor and recipient cells, pHSG299 was transferred approximately 10^4 times more frequently than pHSG399. This result suggested that pHSG299 may contain a specific DNA sequence that promotes cell-to-cell transformation. Therefore we tested this possibility in the following experiments.

3.2. Activities of pHSG399 derivatives containing pHSG299 fragments

By comparing the DNA sequence of pHSG299 with that of pHSG399, sequences unique to pHSG299 that contained a *kan*^{r} gene were identified. To demonstrate the existence of the promoting sequence in pHSG299, six pHSG399 derivatives (pHSG399-F1–F6) were constructed by inserting six pHSG299-specific sequences (F1–F6; approximately 180–370 bp each; Supplementary Table S1) into the multicloning site of pHSG399. Five F1–F5 sequences corresponded to the *kan*^{r} gene and its flanking regions (corresponding to nucleotide positions 2658–1230 in pHSG299). The F6 sequence (no. 1660–1858 in pHSG299) was located in the region between the *lacZα* gene and the pMB1 ori, where no functional genes and elements have been identified. Fig. 1A shows the activities of those pHSG399 derivatives in cell-to-cell transformation. Among them, only pHSG399-F6 clearly showed high activity, indicating that the F6 fragment of pHSG299 contains the promoting sequence.

3.3. Effect of the removal of the F6 sequence from pHSG299 and that of the introduction of the F6 sequence into pUC19

To confirm the above hypothesis, two experiments were performed. One was the removal of the F6 sequence from pHSG299 (Fig. 1A; pHSG299ΔF6). This removal abolished the high transformability of pHSG299. The other was the introduction of the F6 sequence into another plasmid, pUC19-tet (Fig. 1B; pUC19-tet-F6). This introduction caused an increase of about 10^2-fold in the frequency of cell-to-cell transformation. These results confirmed the hypothesis that the F6 sequence of pHSG299 contains the sequence promoting cell-to-cell transformation.

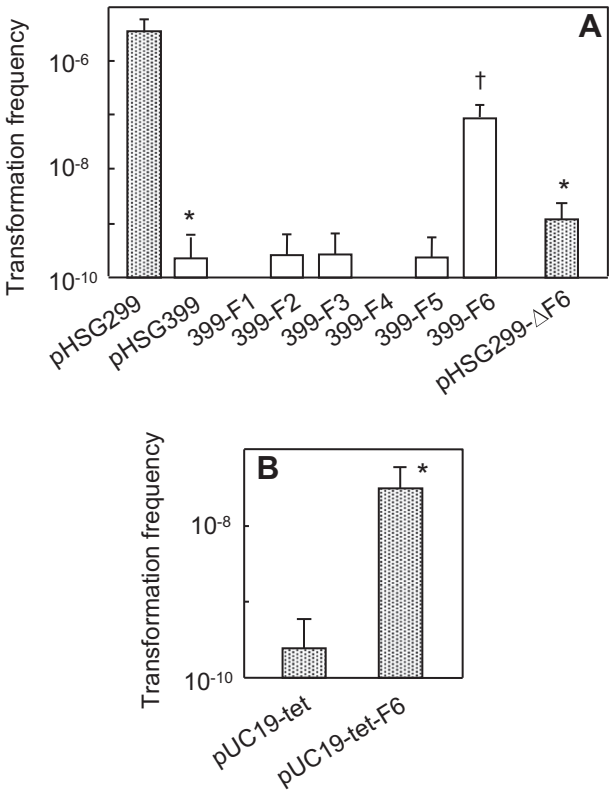

Fig. 1. Frequency of cell-to-cell transformation using pHSG299, pHSG399, and their insertion and deletion derivatives (A) and using pUC19 derivatives (B). Data are mean and S.D. (*t-test, $P < 0.05$, $n = 4$, compared with pHSG299; †t-test, $P < 0.05$, $n = 4$, compared with pHSG399).

3.4. Activity of the F6 sequence in other types of transformation

The activity of the F6 sequence in other types of transformation in *E. coli* was examined (Fig. 2). Previously, we [20] and others [16,18] demonstrated that *E. coli* developed modest natural competence in a colony biofilm. Therefore, we examined the effect of the F6 sequence on natural transformation with purified plasmids in a colony biofilm (Fig. 2A). However, neither pHSG299 nor pHSG399-F6 showed high activity compared with pHSG399. Fig. 2B and C show the results of artificial transformation (Fig. 2B, conventional $CaCl_2$; Fig. 2C, PEG method). Moreover, in these experiments, the F6 sequence did not have any promoting activity in pHSG399. These results suggested that the promoting activity of the F6 sequence is specific to cell-to-cell transformation and is ineffective in other types of natural and artificial transformation.

3.5. Dissection of F6 sequence

To further dissect the 199 bp of the F6 sequence, stepwise deletion analyses were performed (Fig. 3; Supplementary Table S2). Fig. 3A shows the result of the directional deletion of the F6 sequence. This analysis revealed that an 88 bp portion of the F6 sequence (pHSG299 position nos. 1748–1835) possessed full

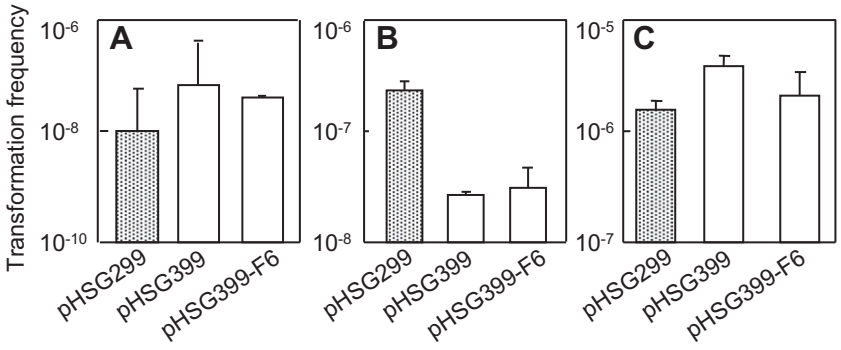

Fig. 2. Frequency of natural transformation (A) and artificial transformation (B and C) using purified pHSG299, pHSG399, and pHSG399-F6. (A) Natural transformation in colony biofilm. (B) Artificial transformation by the $CaCl_2$ method. (C) Artificial transformation by the PEG method. Data are mean and S.D. ($n = 4$).

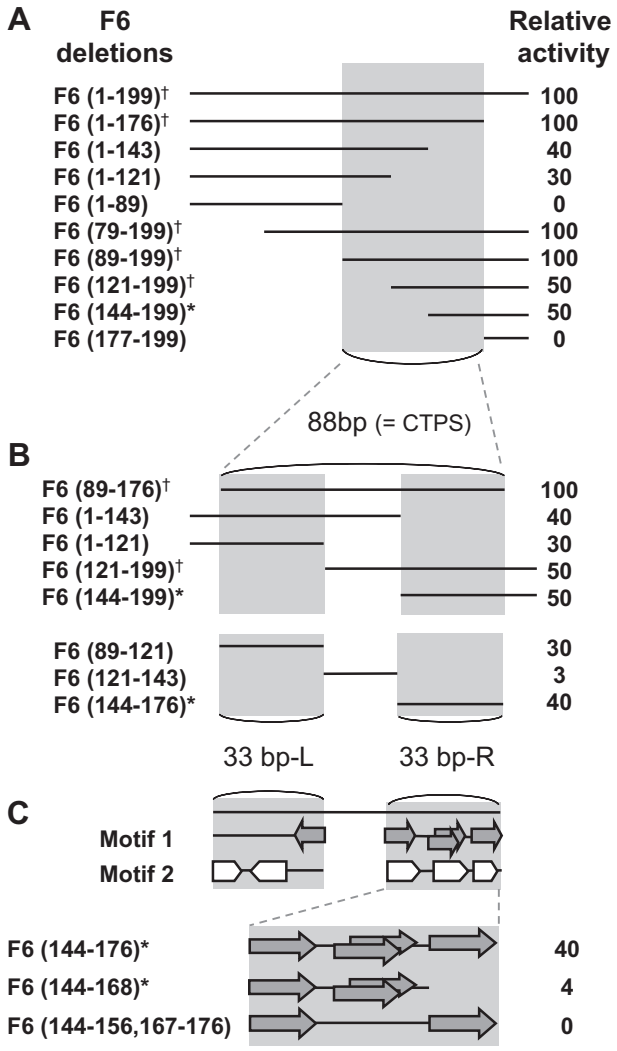

Fig. 3. Activities of the deletion and base substitution mutants of pHSG399-F6 in cell-to-cell transformation. (A) Results of the directional deletion of the F6 sequence. (B) Determination of minimal functional sequences. (C) Analysis of motif sequences. Gray arrows, 5′-AAAACGCTG-3′ motif; white boxes, 5′-A/T_{3-5}G/C_{3-5}-3′ motif. The activity of each mutant in cell-to-cell transformation is presented as the value relative to full-length F6 (1–199), i.e., 100. Each datum was a round number (one significant digit) calculated from the mean (n = 3–5) of the transformation frequency of each plasmid.

promoting activity. We termed this 88 bp sequence as the "cell-to-cell transformation promoting sequence" (CTPS; Accession number: AB634455).

During the sequencing analysis of F6 mutants, we found that the base T in CTPS (position no. 61 in CTPS [or no. 1808 in pHSG299]) differed from the base C in the pHSG299 sequence published in GenBank. This base inconsistency was confirmed by the information obtained from a commercial supplier of pHSG299 (http://catalog.takara-bio.co.jp/en/PDFFiles/3299_DS_e.pdf). Therefore, we considered that this base T commonly existed in the distributed pHSG299 plasmid. Because we designed PCR primers for the construction of F6 deletion mutants based on the sequence data in GenBank, several F6 mutants had the base C while the others had the base T in this position (derivatives possessing T are marked with an asterisk (*) and those possessing C with a dagger (†) in Fig. 3). However, by comparison of mutants (Fig. 3A; F6 (121–199) vs. F6 (144–199)), we estimated that the T/C difference had little effect on activity.

Further analysis of the results of deletion mutants (Fig. 3B) revealed that the CTPS comprises two active elements (33 bp-L and 33 bp-R), each of which consists of a 33 bp sequence and can function independently with partial (approximately 1/3rd) activity (F6 (89–121) and F6 (144–176)).

3.6. Analysis of two 33 bp sequences in F6 sequence

By analysis of the two 33 bp sequences, we identified two candidates for the sequence motif (Figs. 3C and 4A). One was a (5′-AAAACGCTG-3′) motif (gray arrows in Fig. 3C and dashed arrows (– –>) in Fig. 4A), and the other is a less specific (5′-A/T_{3-5}G/C_{3-5}-3′) motif (white boxes in Fig. 3C and dashed arrows (⟹) in Fig. 4A). These motifs overlapped in 33 bp-R, but not in 33 bp-L. Two possible sequences of the former motif in the middle of 33 bp-R overlapped with each other (Figs. 3C and 4A).

To further dissect one of the 33 bp sequences (33 bp-R) and ascertain the importance of the (5′-AAAACGCTG-3′) motif (including the (5′-A/T_{3-5}G/C_{3-5}-3′) motif), deletion and base substitution mutants, which lacked one motif or the two overlapping motifs in 33 bp-R, were constructed [Fig. 3C; F6 (144–168) and F6 (144–156, 167–176)]. We observed that both mutants almost lost activity. This result suggested that the 33 bp sequence may be a minimum functional unit as a promoting sequence, and sequences containing the (5′-AAAACGCTG-3′) motif may be important for the promoting function. To reconfirm the influence of the aforementioned T/C variation (position no. 61 in the CTPS), we constructed T and C variants of 33 bp-R [pHSG399-F6 (144–176, 149T) and pHSG399-F6 (144–176, 149C) in Supplementary Table S2], maintaining the remaining sequences identical with each other. However, no clear difference in CTPS activity was observed between them (data not shown). This result suggests that this variation is not critical for CTPS activity.

We searched the GenBank and BLAST databases for this (5′-AAAACGCTG-3′) motif, but did not find an identical motif. Instead, among the reports on *E. coli* transformation, we found that an 18 bp sequence (5′-CTAGCGCGTTTAAATAGC-3′) reported by Tsen et al. [16] contained a 9 bp sequence (5′-AAACGCGCT-3′; complementary to the sequence underlined above) identical to a part of 33 bp-R (Fig. 4). Since Tsen et al. suggested that this 18 bp sequence could facilitate natural transformation in *E. coli*, the ability of this sequence to promote cell-to-cell transformation was examined (plasmids in Supplementary Table S3). However, this sequence showed no promoting activity in either orientation (data not shown). Furthermore, five other sequences of Tsen (5′-GAAGGAAGATCTTCACCGT-3′, 5′-GGTCGCAAGAGTCTTTGTAC-3′, 5′-CTCCGAGAGATAGAAGCA-3′, 5′-TTTCCACCCTAGACTCGTAC-3′ and 5′-TTTCCCACCCTAGACTCGTA-3′) [16] showed no promoting activity (Supplementary Table S3; data not shown). Therefore, these results suggested that the sequences reported by Tsen et al. do not possess CTPS activity.

4. Discussion

This present study is the first to identify that the specific 88 bp DNA sequence CTPS, derived from a general cloning vector, pHSG299, can function as a DNA element promoting cell-to-cell transformation in *E. coli*. Although full activity was exhibited by the 88 bp sequence, partial activity was exhibited by two individual 33 bp sequences in the 88 bp sequence (Figs. 1 and 3). All the results shown here were obtained in a colony biofilm setting; however, a similar promoting effect of CTPS was observed in a liquid culture setting (data not shown), consistent with a previous study [24].

In the pHSG299 sequence, CTPS is located between the *lacZα* gene and the pMB1 ori, where no functional genes and elements have been identified. A sequence identical to CTPS is often found in the BLAST database among the general cloning vector sequences,

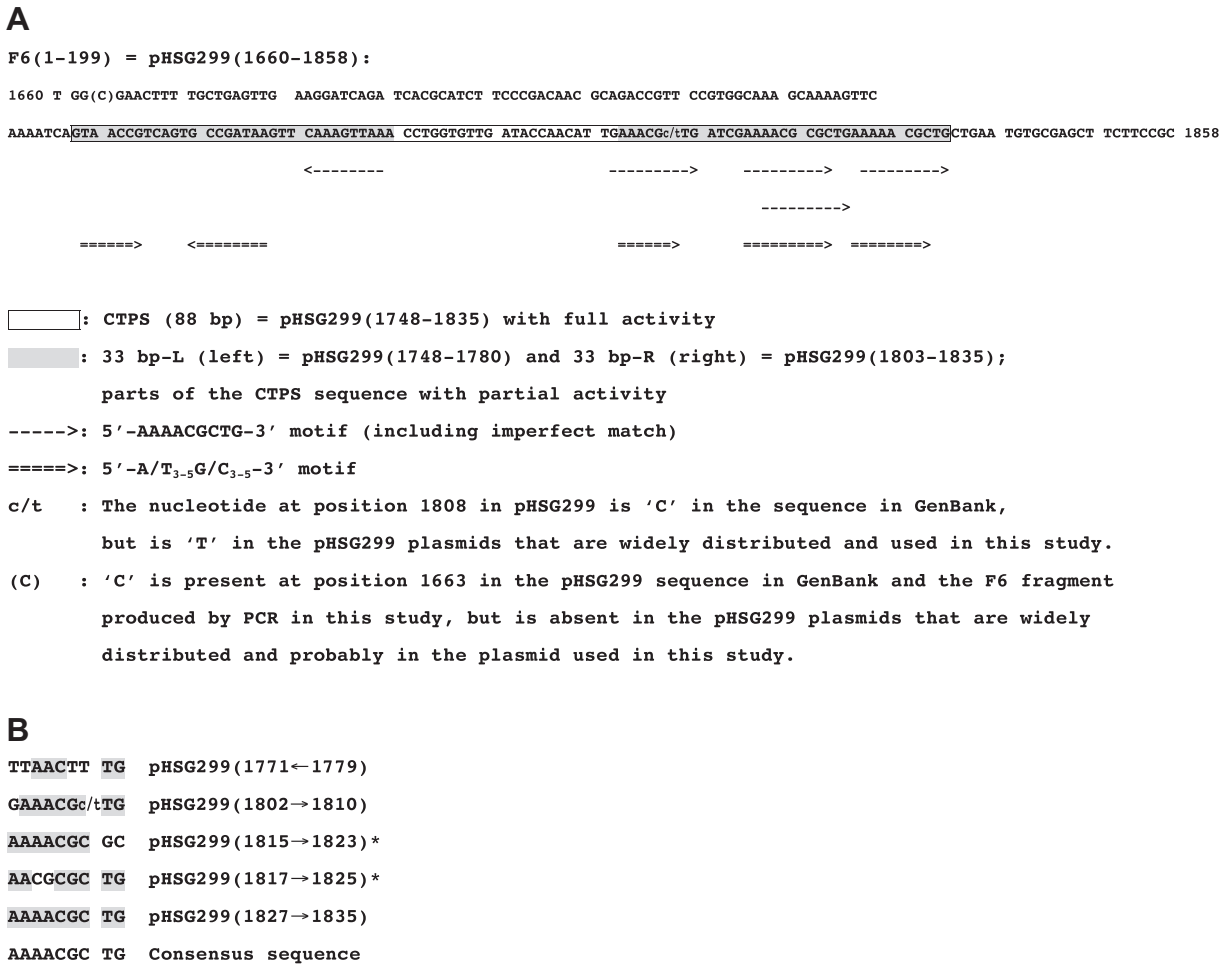

Fig. 4. Primary structures of F6, cell-to-cell transformation promoting sequence (CTPS), 33 bp-L and 33 bp-R. (A) Sequences of F6 and CTPS. Notes for CTPS, 33 bp-L, 33 bp-R, putative sequence motifs (arrows), and the bases to note are also shown. (B) Comparison of five possible 5′-AAAACGCTG-3′ motifs. Two sequences with asterisks (*) overlap with each other. Consensus bases are shown by gray background.

but its native origin was not found in the BLAST and other databases. By tracing back the construction process of pHSG299 [25,27,28], however, we estimated that CTPS originated from R6-5, a conjugative R plasmid. It is supposed that CTPS was transferred to pHSG299 and other cloning vectors as the part of a restriction-enzyme fragment of R6-5. Although several functional genes and elements (including those related to conjugation) in R6-5 were identified and sequenced [29–31], the entire nucleotide sequence of R6-5 has not been determined until date. However, CTPS is not included among the identified genes and sequences in R6-5 and our previous data [24] indicates that cell-to-cell transformation is clearly distinguishable from conjugative DNA transfer; these facts suggest that CTPS is not involved in the known R6-5 functions including conjugation. CTPS and similar DNA elements may contribute to the horizontal transfer of R and other plasmids by cell-to-cell transformation in the environment.

In *E. coli*, no specific DNA sequence promoting artificial transformation has been reported. For natural transformation, a report by Tsen et al. [16] described that several short (approximately 20 bp) sequences showed promoting activity. However, in the present study, we ascertained that none of the sequences of Tsen et al. that we tested showed CTPS activity. Therefore, we hypothesize that cell-to-cell transformation occurs through a unique transformation mechanism that differs from the known types of transformation, as suggested previously [24]. The results of artificial and natural transformation experiments using CTPS (Fig. 2) confirmed this hypothesis. Therefore, CTPS appears to be a novel functional sequence involved exclusively in cell-to-cell transformation.

In some other Gram-negative bacteria, several specific short sequences (approximately 9–12 bp) promoting natural transformation were reported, such as DUS in *Neisseria* sp. (5′-(AT)GCCGTCTGAA-3′) and USS in *H. influenzae* (5′-AAGTGCGGT-3′) [5–8]. The pilus or a related structure on the cell surface may recognize and bind to DUS or USS [5]. This process is postulated to assist the transport of DNA across the outer membrane. No sequences identical to or highly homologous to DUS or USS were found in CTPS. Therefore, it is unclear whether CTPS has a function similar to DUS or USS. However, minimum sequences displaying CTPS activity are short (33 bp) and contain motif-like repetitive sequences (5′-AAAACGCTG-3′ or 5′-A/T_{3-5}G/C_{3-5}-3′); this leads us to hypothesize that certain sequence-specific DNA-binding protein(s) target and bind to these sequences, thereby facilitating cell-to-cell transformation in a process similar to that involving DUS or USS. However, no known protein-binding motifs are found in these 33 bp sequences or in the complete 88 bp CTPS. Thus, an unidentified protein may recognize and bind to CTPS. In addition, we hypothesize, on the basis of the repetitive alignment of the motifs in CTPS (Fig. 4A) and the significant loss of activity resulting from deletion or base substitution of one of the motifs (or two overlapping motifs) (Fig. 3C), that multiple arrangement of the motifs in close proximity may be essential

for these elements to work. Further analyses will be required to reveal whether a DNA-binding protein for CTPS exists and which mechanism involving CTPS promotes cell-to-cell transformation in *E. coli*.

Acknowledgments

This work was supported by a Grant-In-Aid for Scientific Research (No. 19580089) from the Japan Society for the Promotion of Science and in part by a grant from the Institute for Fermentation, Osaka, Japan. We thank Ayuka Sekoguchi for technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.05.040.

References

[1] Lorenz, M.G. and Wackernagel, W. (1994) Bacterial gene transfer by natural transformation in the environment. Microbiol. Rev. 58, 563–602.
[2] Bushman, F. (2002) Lateral DNA Transfer, Cold Spring Harbor Laboratory Press, Plainview, NY.
[3] Thomas, C.M. and Nielsen, K.M. (2005) Mechanisms of and barriers to horizontal gene transfer between bacteria. Nat. Rev. Microbiol. 9, 711–721.
[4] Dubnau, D. (1999) DNA uptake in bacteria. Annu. Rev. Microbiol. 53, 217–244.
[5] Chen, I. and Dubnau, D. (2004) DNA uptake during bacterial transformation. Nat. Rev. Microbiol. 3, 241–249.
[6] Ekins, C., Thomas, C.E., Seifert, H.S. and Sparfling, P.F. (1991) Species-specific uptake of DNA by gonococci is mediated by a 10-base-pair sequence. J. Bacteriol. 173, 3911–3913.
[7] Ambur, O.H., Frye, S.A. and Tønjum, T. (2007) New functional identity for the DNA uptake sequence in transformation and its presence in transcriptional terminators. J. Bacteriol. 189, 2077–2085.
[8] Danner, D.B., Deich, R.A., Sisco, K.L. and Smith, H.O. (1980) An eleven-base-pair sequence determines the specificity of DNA uptake in *Haemophilus* transformation. Gene 11, 311–318.
[9] Mandel, M. and Higa, A. (1970) Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. 53, 159–162.
[10] Hanahan, D. (1983) Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166, 557–580.
[11] Chung, C.T., Niemera, S.L. and Miller, R.H. (1989) One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. USA 86, 2172–2175.
[12] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor Laboratory Press, Plainview, NY.
[13] Baur, B., Hanselmann, K., Schlimme, W. and Jenni, B. (1996) Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. Appl. Environ. Microbiol. 62, 3673–3678.
[14] Bauer, F., Hertel, C. and Hammes, W.P. (1999) Transformation of *Escherichia coli* in foodstuffs. Syst. Appl. Microbiol. 22, 161–168.
[15] Finkel, S.E. and Kolter, R. (2001) DNA as a nutrient: novel role for bacterial competence gene homologs. J. Bacteriol. 183, 6288–6293.
[16] Tsen, S.D., Fang, S.S., Chen, M.J., Chien, J.Y., Lee, C.C. and Tsen, D.H. (2002) Natural plasmid transformation in *Escherichia coli*. J. Biomed. Sci. 9, 246–252.
[17] Woegerbauer, M., Jenni, B., Thalhammer, F., Graninger, W. and Burgmann, M. (2002) Natural genetic transformation of clinical isolates of *Escherichia coli* in urine and water. Appl. Environ. Microbiol. 68, 440–443.
[18] Sun, D., Zhang, Y., Mei, Y., Jiang, H., Xie, Z., Liu, H., Chen, X. and Shen, P. (2006) *Escherichia coli* is naturally transformable in a novel transformation system. FEMS Microbiol. Lett. 265, 249–255.
[19] Maeda, S., Kakihara, N. and Koishi, Y. (2003) Competency development of *Escherichia coli* in foodstuffs. Microbes Environ. 18, 100–103.
[20] Maeda, S., Sawamura, A. and Matsuda, A. (2004) Transformation of colonial *Escherichia coli* on solid media. FEMS Microbiol. Lett. 236, 61–64.
[21] Ishimoto, Y., Kato, S. and Maeda, S. (2008) Freeze-thaw-induced lateral transfer of non-conjugative plasmids by in situ transformation in *Escherichia coli* in natural waters and food extracts. World J. Microbiol. Biotechnol. 24, 2731–2735.
[22] Maeda, S., Ito, M., Ando, T., Ishimoto, Y., Fujisawa, Y., Takahashi, H., Matsuda, A., Sawamura, A. and Kato, S. (2006) Horizontal transfer of nonconjugative plasmids in a colony biofilm of *Escherichia coli*. FEMS Microbiol. Lett. 255, 115–120.
[23] Ando, T., Itakura, S., Uchii, K., Sobue, R. and Maeda, S. (2009) Horizontal transfer of non-conjugative plasmid in colony biofilm of *Escherichia coli* on food-based media. World J. Microbiol. Biotechnol. 25, 1865–1869.
[24] Etchuuya, R., Ito, M., Kitano, S., Shigi, F., Sobue, R. and Maeda, S. (2011) Cell-to-cell transformation in *Escherichia coli*: a novel type of natural transformation involving cell-derived DNA and a putative promoting pheromone. PLoS ONE 6 (1), e16355.
[25] Takeshita, S., Sato, M., Toba, M., Masahashi, W. and Hashimoto-Gotoh, T. (1987) High-copy-number and low-copy-number plasmid vectors for lacZα-complementation and chloramphenicol- or kanamycin-resistance selection. Gene 61, 63–74.
[26] Singer, M., Baker, T.A., Schnitzler, G., Deischel, S.M., Goel, M., Dove, W., Jaacks, K.J., Grossman, A.D., Erickson, J.W. and Gross, C.A. (1989) A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. 53, 6408–6411.
[27] Brady, G., Jantzen, H.M., Bernard, H.U., Brown, R., Schütz, G. and Hashimoto-Gotoh, T. (1984) New cosmid vectors developed for eukaryotic DNA cloning. Gene 27, 223–232.
[28] Hashimoto-Gotoh, T., Franklin, F.C., Nordheim, A. and Timmis, K.N. (1981) Specific-purpose plasmid cloning vectors. I. Low copy number, temperature-sensitive, mobilization-defective pSC101-derived containment vectors. Gene 16, 227–235.
[29] Bagdasarian, M., Bailone, A., Bagdasarian, M.M., Manning, P.A., Lurz, R., Timmis, K.N. and Devoret, R. (1986) An inhibitor of SOS induction, specified by a plasmid locus in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 83, 5723–5726.
[30] Loh, S., Skurray, R., Célérier, J., Bagdasarian, M., Bailone, A. and Devoret, R. (1990) Nucleotide sequence of the psiA (plasmid SOS inhibition) gene located on the leading region of plasmids F and R6-5. Nucleic Acids Res. 18, 4597.
[31] Cram, D.S., Loh, S.M., Cheah, K.C. and Skurray, R.A. (1991) Sequence and conservation of genes at the distal end of the transfer region on plasmids F and R6-5. Gene 104, 85–90.